PATENT SPECIFICATION

(11) 1 437 458

(21) Application No. 21398/73 (22) Filed 4 May 1973
(44) Complete Specification published 26 May 1976
(51) INT CL² G01N 33/18//27/26
(52) Index at acceptance G1B 13A1 13D2 17X1 18A 22D 22L
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(54) CONTINUOUS DETERMINATION OF THE TOXICITY OF LIQUIDS

(71) We, BROWN, BOVERI & CIE AKTIENGESELLSCHAFT, a German Company, of 6800 Mannheim 1, Postfach 351, Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to a method for the continuous determination of the overall toxicity of water, sewage, and other liqids by means of micro-organisms and to apparatus for carrying out the method.

It is known that the action of toxic agents upon micro-organisms (bacteria, algae, protozoa) runs parallel with the action thereof upon higher animals and human beings, so that micro-organisms may be used instead of higher animals for numerous non-specific toxicity tests in vivo.

Methods for testing the harmfulness to rivers and lakes or sewage or waste water of unknown composition by determining the injurious effect upon some micro-organisms, typical of the draining ditch or canal, have also been worked out and described. Whilst the results of tests carried out by the known test methods permit, in principle, the drawing of inferences therefrom about the toxic effect upon higher animals and human beings, the drawing of such inferences is actually not intended. Apart therefrom, these methods have a few disadvantages which have hitherto impeded their wider use. Their operation is protracted and expensive and the convincing power of the evidence obtained is limited by the difficulties encountered in the breeding (culturing) and storage of micro-organisms of adequately reproducible properties. In professional circles these difficulties are given such weight as to lead to the general assumption that the use of micro-organisms as a test technique with the normal requirements of reproducibility, possibility of calibration and insusceptibility to trouble is impracticable.

Based on these considerations, it is an object of the invention to provide a method for continuously determining the toxicity of an aqueous liquid, particularly sewage or other waste water, which is a decisive improvement of the known methods inasmuch as the use of micro-organisms enables it to give sufficiently reproducible results and to be carried out in continuous operation.

In accordance with the invention, there is provided a method of continuously measuring the toxicity of an aqueous liquid, which comprises maintaining in a stock vessel a constant concentration of a specific micro-organism or a specific mixture of micro-organisms by continuously cultivating the micro-organism or mixture of micro-organisms under constant conditions in the stock vessel with a constant inflow of water comprising nutrients into the stock vessel and an outflow of culture solution from the stock vessel equal to the total liquid inflow into the stock vessel, passing the outflowing culture solution into a measuring vessel in which the culture solution is mixed for a residence time of less than the period of procreation of the micro-organism or mixture of micro-organisms with the aqueous liquid being tested and with fresh nutrient solution, the fresh nutrient solution and the aqueous liquid being saturated with atmospheric oxygen and being also passed into the measuring vessel at constant rate, and, as an indicator of the toxicity of the aqueous liquid being tested, measuring a parameter dependent on the metabolic activity of the micro-organism or mixture of micro-organisms in the measuring vessel. In many cases, the turbidity or a change in colour may, for example, serve as the parameter or variable which is measured. However, parameters the measurement of which is more universally applicable include (i) the pH, (ii) the carbon dioxide evolved by the oxidation processes which occur, and (iii) the oxygen concentration, the latter being the parameter which is preferably measured. It has to be borne in mind that

poisoning of the micro-organisms in the measuring vessel results in a reduced carbon dioxide concentration or in an increased oxygen content (reduced oxygen consumption).

The method according to the invention is distinguished from the known test methods referred to primarily by the fact that it is suitable for the continuous determination of test data, decisively improves the reproducibility of the properties of the micro-organisms used and decisively simplifies the breeding and storage thereof.

In accordance with the invention, the breeding or cultivating of the micro-organisms proceeds in the stock vessel to which determined quantities of specific nutrients for the micro-organism or micro-organisms to be cultured are substantially continuously fed at a constant temperature. By intensive agitation and free access of air, the one or more micro-organisms (with which the stock vessel has previously been inoculated) as well as their products of metabolism and any undissolved nutrient present are maintained in constant suspension and there is a vigorous exchange of gas between the atmosphere and the soltuion, thereby ensuring an adequate supply of oxygen to the micro-organisms and the removal of the carbon dioxide (CO_2) gas evolved.

Flow systems of this kind, like any other constantly charged flow systems, tend towards a stationary state of equilibrium, in which types and concentrations of the chemical substances and micro-organisms are and remain constant. The number of types of micro-organisms is limited the more, and their properties are defined the more sharply, the more precisely controlled the total chemical and phsyical conditions (composition and concentration of the nutrients, composition and concentration of the products of metabolism including carbon dioxide (CO_2), the oxygen (O_2) concentration, and the temperature).

The precision of control of these environmental conditions, disregarding the temperature, in a stationary state of equilibrium of a flow system is much superior to that obtainable in the closed systems normally used for breeding or culturing micro-organisms. This advantage is decisively increased, since by fixing the dimensions of the vessel and the rate of flow, the average resident time of the liquid in the stock vessel is precisely defined and adjusted in relation to the period of procreation or generation of the micro-organisms to be bred or cultured. The adjustment is so made as to give the said average residence time a value which, while distinctly exceeding the procreation period in the exponential phase of propagation, preferably still remains in the same order of magnitude.

An average residence time which is equal to a precreation period slightly beyond the exponential propagation phase, is, for theoretical reasons, an indispensable prerequisite to a permanent existence of the micro-organisms in a stationary state of equilibrium. In this state of the flow system, the surviving types of micro-organisms meet the condition:— effective procreation period equals average residence time even under the most adverse environmental conditions with respect to shortage of nutrients and accumulation of metabolism products.

This sharp selectivity enables the solution in the stock vessel to be in constant contact with non-sterile air without the bacteria introduced by the air having any marked influence upon the biological-chemical equilibrium or balance in that solution. By appropriate selection of the nutrient and temperature conditions, the autoselectivity may, if desired, be promoted to such an extent that within a few days a single determined type of bacterium, that is a monoculture, remains in the stock vessel irrespective of whether or not the liquid has originally been inoculated and irrespective of the type of inoculating agent used. In carrying out toxicological tests, a limitation of the number of types is desirable insofar as various types respond differently to different toxic agents. Definitive statements thus presuppose definite types of micro-organisms and, in extreme cases, monocultures.

The nutrients fed into the stock vessel are advantageously in the form of a nutrient-containing solution which is preferably previously prepared and stored in the form of a substantially autosterile concentrate which becomes non-sterile as a result of dilution. The same nutrients as those added to the stock vessel are preferably also added to the the aqueous liquid the toxicity of which is to be measured or tested.

The effluent from the stock vessel, that is the culture solution, is passed into a measuring vessel and is therein mixed with the aqueous liquid being tested. A parameter dependent on the metabolic activity of the micro-organism of mixture of micro-organisms in the culture solution is measured in the measuring vessel as an indicator or measurement of the toxicity of the aqueous liquid being tested.

Apparatus for carrying out the method according to the invention is also provided, the apparatus comprising (a) a stock vessel open to the ambient atmosphere and provided with a stirrer, preferably a mechanically driven agitator, conduits for feeding nutrient solution and diluent water

into the stock vessel, and a discharge conduit, (b) a measuring vessel from which air can be excluded, the measuring vessel being provided with a stirrer, preferably a magnetic stirrer, a first inlet and a second inlet, the first inlet being connected to the discharge conduit of the stock vessel, and means for measuring a parameter dependent on the metabolic activity of a micro-organism or mixture of micro-organisms present in a liquid in the measuring vessel, (c) a preliminary vessel open to the ambient atmosphere, the preliminary vessel being provided with an air supply line and a discharge conduit which is connected to the second inlet of the measuring vessel, and (d) an overflow pipe connected to the measuring vessel, the level of the overflow pipe determining the level of liquids in the stock and preliminary vessels. The said means is preferably one effective to measure the concentration of oxygen in a liquid, particularly an oxygen electrode.

The dimensions of the stock, measuring and preliminary vessels are adjusted to the period of procreation of the micro-organism or mixture of micro-organisms used in the solution in the stock vessel and to the quantity thereof in continuous flow, so that the residence time of the solution in the stock vessel exceeds the period of procreation and the residence time in the measuring vessel is considerably shorter than the period of procreation.

Preferably, the stock vessel and the preliminary vessel are disposed at the same level and are of the same height, and the measuring vessel is disposed at a level lower than the stock and preliminary vessels.

The method according to the invention is hereinafter described with reference to the accompanying drawing in which apparatus suitable for carrying out the method is diagrammatically illustrated.

A stock vessel 1 which is open to the atmosphere and is provided with an agitator or stirrer 21, contains a liquid nutrient or stock solution 22 inoculated with a specific micro-organism or mixture of micro-organisms. The oxygen concentration in the stock solution 22 is maintained substantially at air saturation concentration by vigorous agitation of the solution in the open stock vessel 1. The stock vessel 1 is continuously fed at a constant rate with a concentrated nutrient solution through conduit 23 and with diluent water through conduit 24.

When the stock solution 22 has been inoculated with, for example, Bact.coli. Esch., the concentrated nutrient solution may consist of, for example., 10% peptone, 10% lactose, 20% common salt and 60% water. The handling of the concentrated nutrient solution is facilitated since, owing to its high common salt concentration, the concentrated nutrient solution is substantially autosterile and remains stable in storage for several weeks without undergoing any change even when left to stand unprotected in an open container exposed to non-sterile air. That concentrated solution and the diluent water are fed simultaneously, preferably in a ratio of 1 : 50, into the stock vessel 1 so that the common salt concentration in the stock solution is reduced sufficiently to ensure an unimpeded growth and propagation of the micro-organisms.

The culture solution leaves the stock vessel 1 through discharge conduit 25 at a constant rate which is equal to the total inflow rates of the concentrated nutrient solution and the diluent water. From the conduit 25, the culture solution flows through first inlet 26 into a smaller measuring vessel 2 which is sealed against the access of air and provided with a magnetic stirrer 3, to leave the apparatus through an overflow 4. An oxygen electrode 5 responds in known manner to the oxygen (O_2) concentration in the liquid contained in the measuring vessel 2. The oxygen concentration in the measuring vessel 2 is, as a result of the constant consumption of oxygen through the metabolic activity of the micro-organisms, generally below air saturation concentration.

The aqueous liquid to be tested for its toxicity (being generally waste water or sewage) has also to be saturated with atmosphereic oxygen upon entering the measuring vessel 2, so that errors in measurement may be avoided. For this purpose, the aqueous liquid to be tested is passed initially through conduit 28 into a preliminary vessel 6, in which, unless it is already saturated with air, the oxygen concentration in the test liquid is brought into equilibrium with the atmosphere by bubbling air through the liquid from an air supply line 29. This saturation with oxygen may be effected in another manner, for example by intensive sitrring as in the stock vessel 1. Simultaneously, metered quantities of nutrient solution, advantageously the same nutrient solution as that fed continuously to the stock vessel 1, are added through conduit 30 to the test liquid in the preliminary vessel 6.

The aqueous liquid to be tested, saturated with atmosphereic oxygen, flows continuously at a constant rate from the preliminary vessel 6 through discharge conduit 31 and second inlet 27 into the measuring vessel 2. In the measuring vessel, the aqueous liquid is mixed, by the magnetic stirrer 3, with the culture solution from the stock vessel 1 and the mixture of liquids leave the measuring vessel through the overflow pipe 4.

An important difference between the vessels 1 and 2 is that the average residence time of the liquid in the stock vessel 1 is distinctly longer than, and in the measuring vessel 2 is distinctly shorter than, the period of procreation of the micro-organisms in the exponential propagation phase.

Since, under favourable conditions essential for life, the shortest known period of precreation, that is the period of procreation of Bact.coli. Esch., is about 20 minutes, it is sufficient to maintain a residence time of about 10 or 5 minutes in the measuring vessel 2 to ensure that all of the micro-organisms must leave the vessel at a speed exceeding propagation speed.

The propagation of the micro-organisms in the culture solution flowing continuously from the discharge conduit 25 into the measuring vessel 2 is already slightly inhibited by the self-produced shortage of nutrient, although the micro-organisms are still very young (average age equals average residence time in stock vessel 1). and extremely eager for life. In the measuring vessel 2, they are continuously supplied with fresh nutrient solution through the preliminary vessel 6. This nutrient solution instantaneously stimulates their vital activity, as is evident from a corresponding oxygen (O_2) consumption measured by the oxygen electrode 5 in terms of a reduced oxygen concentration which may be indicated by or read on an indicating or recording attachment. Any factor conditioned by the metabolism (for example an increase in CO_2, or a change in colour or turbidity) may be measured instead of the oxygen consumption.

A test liquid flowing through the preliminary vessel 6 into the measuring vessel 2 contains toxic agents by which the micro-organisms also passing from the stock vessel 1 into the measuring vessel 2 are destroyed or the vital activity thereof is inhibited, this factor being noticable from a reduced, or complete discontinuance of, oxygen consumption in the measuring vessel 2. The oxygen concentration measured in the measuring vessel approaches air saturation concentration and attains substantially total air saturation concentration when the power or concentration of the poison is such as to instantaneously destroy all of the micro-organisms.

The ratio of the rate of flow of the culture solution to the rate of flow of the test liquid as well as the composition and concentration of the nutrient solution in relation to the type and concentration of the micro-organisms, provide the means of fixing the method's senstiveness of measuring. The zero or null position, that is to say the oxygen concentration in the measuring vessel in the absence in the test liquid of any toxic agent which acts upon the micro-organisms, is obtained by using pure water as the test liquid.

Details of the change in oxygen concentration, advantageously measured with the use of Bact.coli. Esch., are given in the following example. The oxygen concentration may be adjusted and maintained, for example, at 20% air saturation, by appropriate selection of the supply of nutrient, the residence times and the mixing ratio of culture solution to test liquid. In the complete absence of toxic agents, this is a preferred value for the end point of the scale.

In this way, an oxygen measuring range of from, preferably 20% to 100% of air saturation may be fixed and associated with a 0 to 100% of poisonous effect. Instead of assuming an arbitrary, for example linear, relationship between the 0—100% poisonous effect of the test liquid and the said oxygen measuring range, a series of dilutions of particularly interesting or relevant toxic agents may be used as test liquids, and the corresponding oxygen concentrations in the measuring vessel determined. The several oxygen concentrations are thus co-ordinated with the concentrations of toxic agents, the whole system being thereby calibrated.

The apparatus becomes a genuine measuring instrument by virtue of this capacity for calibration and by virtue of the fact that two end points of the scale may be readily checked at any time by using non-toxic or highly toxic test water.

WHAT WE CLAIM IS:—

1. A method of continuously measuring the toxicity of an aqueous liquid, which comprises maintaining in a stock vessel a constant concentration of a specific micro-organism or a specific mixture of micro-organisms by continuously cultivating the micro-organism or mixture of micro-organisms under constant conditions in the stock vessel with a constant inflow of water comprising nutrients into the stock vessel and an outflow of culture solution from the stock vessel equal to the total liquid inflow into the stock vessel, passing the outflowing culture solution into a measuring vessel in which the culture solution is mixed for a residence time of less than the period of precreation of the micro-organism or mixture of micro-organisms with the aqueous liquid being tested and with fresh nutrient solution, the fresh nutrient solution and the aqueous liquid being saturated with atmospheric oxygen and being also passed into the measuring vessel at a constant rate, and, as an indicator of the toxicity of the aqueous liquid being tested, measuring a

parameter dependent on the metabolic activity of the micro-organism or mixture of micro-organisms in the measuring vessel.

2. A method according to claim 1, in which said parameter is the oxygen consumption.

3. A method according to claim 1, in which said parameter is the pH.

4. A method according to claim 1, in which said parameter is the carbon dioxide evolved.

5. A method according to claim 1, in which said parameter is the turbidity or change of colour.

6. A method according to any one of the preceding claims, in which the aqueous liquid is sewage or other waste water.

7. A method according to any one of the preceding claims, in which the solution in the stock vessel is subjected to a vigorous exchange of gas with the atmosphere.

8. A method according to any one of the preceding claims, in which the nutrients added to the aqueous liquid the toxicity of which is to be measured, are the same as those fed into the stock vessel.

9. A method according to any one of the preceding claims, in which the nutrients fed into the stock vessel are in the form of a nutrient-containing solution which is prepared and stored in the form of a substantially autosterile concentrate which becomes non-sterile as a result of dilution.

10. A method according to claim 1, substantially as hereinbefore described.

11. Apparatus for carrying out the method according to claim 1, which comprises (a) a stock vessel open to the ambient atmosphere and provided with a stirrer, conduits for feeding nutrient solution and diluent water into the stock vessel, and a discharge conduit, (b) a measuring vessel from which air can be excluded, the measuring vessel being provided with a stirrer, a first inlet and a second inlet, the first inlet being connected to the discharge conduit of the stock vessel, and means for measuring a parameter dependent on the metabolic activity of a micro-organism or mixture of micro-organisms present in a liquid in the measuring vessel, (c) a preliminary vessel open to the ambient atmosphere, the preliminary vessel being provided with an air supply line and a discharge conduit which is connected to the second inlet of the measuring vessel, and (d) an overflow pipe connected to the measuring vessel, the level of the overflow pipe determining the level of liquids in the stock and preliminary vessels.

12. Apparatus according to claim 11, in which the said means is effective to measure the concentration of oxygen in a liquid.

13. Apparatus according to claim 12, in which the said means is an oxygen electrode.

14. Apparatus according to any one of claims 11 to 13, in which the dimensions of the three vessels are adjusted to the period of procreation of the micro-organism or mixture of micro-organisms used in the stock vessel solution and to the quantity thereof in continuous flow, so that the residence time in the stock vessel exceeds the period of procreation and the residence time in the measuring vessel is considerably shorter than the period of procreation.

15 Apparatus according to any one of claims 11 to 14, in which the stock vessel and the preliminary vessel are disposed at the same level and are of the same height, and in which the measuring vessel is disposed at a level lower than the stock and preliminary vessels.

16. Apparatus according to any one of claims 11 to 15, in which the stirrer in the stock vessel is a mechanically driven agitator and the stirrer in the measuring vessel is a magnetic stirrer.

17. Apparatus for carrying out the method claimed in claim 1, substantially as hereinbefore described with reference to the accompanying drawings.

EDWARD EVANS & CO.,

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1976.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.

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1 SHEET *This drawing is a reproduction of the Original on a reduced scale*